chromosomes not only in cancer cells but also in primary IMR90 cells, which are possible causes for genetic instability. Taken together, these observations suggest that KIFC1 is essential for proper chromosome segregation to maintain genomic stability. Yeast two-hybrid search and co-immunoprecipitation of KIFC1 demonstrated that KIFC1 directly interacts with Op18/sthathmin, which regulates microtubule dynamics by inhibiting tubulin polymerization. When KIFC1 and Op18 were doubly knocked downed in MDA-MB-231 cells, the portion of cells with multiple poles and the number of micronuclei was highly increased. These results suggest that KIFC1 functions with Op18 during mitosis.

3563-Pos Board B424

Mitotic Kinesin CENP-E is a Robust Tracker of Dynamic Microtubule ends

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Accurate chromosome segregation during mitosis requires durable linking between kinetochores and plus ends of spindle microtubules. During metaphase the ends of kinetochore microtubules continuously assemble and disassemble but the chromosomes remain stably attached and move concomitantly with microtubule dynamics. So far only Dam1 kinetochore complex has been reported to be able to maintain stable association with dynamic microtubule ends in vitro. The homologous genes, however, are present only in fungi, so the molecular identities of kinetochore-microtubule couplers in other cells are not known. Here, we report that a conserved kinesin-like CENP-E, playing an essential role in chromosome segregation, can maintain long-lasting association with the ends of dynamic microtubules in vitro and couple microtubule depolymerization to the motion of a microbead cargo. Using purified recombinant CENP-E from Xenopus laevis we show that a truncated dimeric motor, which lacks a stalk and a tail, fails to track the dynamic microtubule ends. However, the rate and processivity of the plus-end-directed motility and the response to applied force are similar for the full length and truncated proteins. Interestingly, a walking full length CENP-E has a folded configuration and remains compact even under tension, suggesting that such conformation is important for CENP-E's functioning at the kinetochore. To examine whether the 230nm-long CENP-E stalk is important for kinetochore-microtubule interactions, we used siRNA-mediated depletion of endogenous CENP-E in cells with stable expression of a truncated "bonsai" version of CENP-E, which localizes normally to kinetochores. In cells with "bonsai" CENP-E the chromosome congression was delayed. Furthermore, the aligned chromosomes showed marked instability and frequently moved away from the metaphase plate, implying defects in kinetochore-microtubule attachment. We propose that CENP-E exerts some of its mitotic functions via its non-motor domains, which enable it to track dynamic microtubule ends.

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Dynein Function in T Cell MTOC Translocation and Vesicle Movements

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Binding of T cells to antigen-presenting cells triggers translocation of the T cell microtubule organizing center (MTOC) and secretory vesicles to the immunological synapse, processes that are essential for T cell effector function. Data from localization and functional studies indicate that MTOC translocation and secretory vesicle movement rely on discrete pools of dynein. For example, we show that siRNA knockdown of dynactin blocks secretory vesicle accumulation but not MTOC translocation. In order to dissect how dynein is involved, we used *in vivo* molecular trap technology to sequester dynein intermediate and light chains (DIC and DLCs, respectively). In Jurkat cells expressing the traps, we demonstrated that the target protein co-immunoprecipitated with the trap in the presence but not absence of AP20187 (Ariad). In scoring MTOC translocation in activated Jurkat-Target pairs, addition of AP20187 reduced the values to 37% of positive controls in cells expressing the DIC trap and to 52% of control values in cells expressing the DLC LC8 trap. No effect was observed with the DLC TcTex trap, with or without the drug. Finally, we show that Lissencephaly1 protein (Lis1), a dynein-binding protein that is involved in polarized nuclear movements and spindle orientation, also appears to be important for MTOC polarization. Lis1 is recruited to the immunological synapse and co-immunoprecipitates with dynein. To test whether Lis1 is important for MTOC translocation, we prepared truncated Lis1 constructs containing GFP linked to either the N-terminal homodimerization domain or the C-terminal WD repeat domain. In activated Jurkat-Target pairs, MTOC translocation was reduced to 26% of the positive controls in Jurkat cells expressing the N-terminal domain and to 10% of the positive controls in cells expressing the WD repeat domain. Ongoing studies aim to determine the impact of Lis1 and DLCs on vesicle movements.

Cell & Bacterial Mechanics & Motility IV

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Analysis of E-Cadherin as a Tension Sensor at Epithelial Cell-Cell Junctions

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Cells are mechanosensitive systems in which the detection of external mechanical forces by proteins that span the plasma membrane leads to changes in cell organization, differentiation and proliferation. Cell-cell contacts are thought to be sites of mechano-transduction, and a major cell adhesion protein E-cadherin is a candidate protein to transduce forces between cells under tension. To directly test whether force transduction occurs through E-cadherin, we inserted a force sensor module into the E-cadherin cytoplasmic domain. The force sensor (TSMod) is a 32-aa-long flexible peptide capped by mTFP and Venus that together form a FRET pair. FRET efficiency scales inversely with linker extension, and thus reports the tension exerted on the sensor within the host protein. Using FRET intensity ratio imaging in live MDCK cells, we show that the E-cadherin/TSmod construct is recruited to cell-cell contacts like its endogenous counterpart. We observe decreased FRET both at cell-cell contacts and at the plasma membrane distant from intercellular junctions, indicating that the E-cadherin cytoplasmic domain is under tension regardless of its recruitment to intercellular contacts. Significantly, we observe increased FRET upon actin cytoskeleton disruption or depletion of alpha-catenin, a putative linker between actin and E-cadherin, regardless of subcellular location. These results indicate that the E-cadherin complex is under tension through the catenin complex and actin cytoskeleton, and that this is a constitutive state that is independent of cell-cell adhesion.

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Cells Sense Rigidity of their Micro-Environment by a Unique Force-Sensing Displacement Cassette

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Eukaryotic cells modulate their gene expression profile and differentiation in response to physical cues in their micro environment. The exact mechanism by which the cells sense the stiffness of the substrate has not yet been completely understood. Recent studies with micropillar arrays indicate that cells move fibronectin pillars for a constant displacement irrespective of the stiffness. Thus, rigidity sensing may involve the integration of the force required. To further understand the mechanism involved, we increased the center-to-center spacing of the micropillars to 3 micrometers and compared two different cell lines. We observed that the displacements of the pillars were constant over an order of magnitude variation in stiffness of pillars and a six-fold variation in spacing; however, the mouse embryonic fibroblast (RPTPα+/+) and cells foreskin fibroblast (HFF) cells exerted consistent peak pillar displacements of ~70nm and ~120nm respectively. Chemical and genetic perturbations of likely components of the mechanosensing machinery are being used to probe its composition and working. Delineating the components of this cassette is crucial to understand the sensing of the substrate stiffness by cells with implications for cell differentiation and metastasis.

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Influence of Substrate Stiffness on Cell Spread Area

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It is known that various cell types can sense and respond to the mechanical properties of their microenvironment. Specifically, cells have been known to spread more when cultured on stiff substrates and are able to match their